EMBRYO AND SEEDLING OF DIOON SPINULOSUM

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(WITH PLATES X, XI)

Dioon spinulosum Dyer, imperfectly and incompletely described by EICHLER[1] in 1883, and by DYER[2] in 1885, but fully and carefully by CHAMBERLAIN[3] in 1909, is, like the other 2 species of *Dioon*, endemic in Mexico. The embryos and seedlings which furnish the material for this study were grown from ovules collected by Dr. CHAMBERLAIN in the mountains about Tierra Blanca and Tuxtepec during his several trips to the *Dioon* country. The tree is described by him as a magnificent ornamental cycad 30–40 ft. high. Unlike *D. edule*, it grows rapidly, and in 2 years makes a handsome greenhouse plant with a crown of large, fernlike leaves.

The unique appearance of the plant and the great size of its ovulate strobilus and ovules led to the expectation of great peculiarities in its vascular anatomy and cotyledonary arrangements. The investigation has verified these expectations only in part. The study of the vascular anatomy of the embryo and seedling of *D. spinulosum* merely serves to emphasize the general harmony which prevails among the cycads in this respect.

Embryo

The seed, like those of all cycads, is filled with a massive endosperm stored with starch. Upon this tissue the proembryo and embryo proper are nourished, apparently without any resting period. When the embryo has attained a length equal to that of the seed itself, pressure is exerted upon the stony coat, and the thin region near the micropyle is broken. Fig. 1 shows a seed with a young embryo borne on the twisted suspensor; fig. 2 represents

[1] EICHLER, A. W., Ein neues *Dioon*. Gartenflora 2:411. 1883.

[2] DYER, SIR W. T. THISTLETON, Biologia Centrali Americana, Botany 3:190. 1885.

[3] CHAMBERLAIN, C. J., *Dioon spinulosum*. BOT. GAZ. 48:401–413. 1909.

a later stage in which the embryo has attained its full length, has broken the seed coat, and pushed out the dried remains of the suspensor and archegonial wall.

The embryo at this stage consists of cotyledons, plumule, and a basal part which in its upper portion is hypocotyl, and in its lower portion root sheath; for the root is endogenous, and is not preceded by any structure which might be looked upon as a "radicle" or "caulicle." The hypocotyl is extremely short, and the distinction between it and the root sheath cannot be determined superficially, but only by study of the internal structure. The number of cotyledons varies from 2 to 4, and all stages of their union are represented (figs. 20–22, 24).

The vascular cylinder of the hypocotyl is a protostele. It has 4 easily recognized protoxylem groups, in no way differing from the hypocotyl cylinders of *Ceratozamia*[4] and *Microcycas*.[5] The cotyledons are multifascicular, like those of *Ceratozamia* and *Microcycas*, and unlike those of *Zamia* and *Dioon edule*. In the embryos which are dicotyledonous, the manner in which the cotyledonary traces are supplied from the hypocotyl cylinder is exactly the same as that described by THIESSEN[6] for *Dioon edule*, and which COULTER and CHAMBERLAIN[7] have shown to be characteristic of the cycads. When the embryo has 4 cotyledons, each cotyledon is on a side of the quadrangular node, and receives a secondary bundle from each of the adjacent angles. Twelve out of 100 embryos had 4 cotyledons, and in each case the vascular strands arose in this manner. In the tricotyledonous embryos (there were 4 of them in 100), one of the 3 cotyledons was supplied in the dicotyledonary manner, and the other two after the manner of the embryos with 4 cotyledons; and yet, at a level just above the tip of the plumule, the number of vascular strands was about equal in the 3 cotyledons. The strands

[4] DORETY, HELEN A., The seedling of *Ceratozamia*. BOT. GAZ. 46:203–220. *pls. 12–16*. 1908.

[5] ———, Vascular anatomy of the seedling of *Microcycas calocoma*. BOT. GAZ. 47:139–147. *pls. 5, 6*. 1909.

[6] THIESSEN, REINHARDT, The vascular anatomy of the seedling of *Dioon edule*. BOT. GAZ. 46:357–380. *pls. 23–29*. 1908.

[7] COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of gymnosperms. Chicago. 1910.

are endarch where they separate from the hypocotyl cylinder (fig. 28); they become mesarch just after they enter the base of the cotyledon (fig. 27) and maintain that character throughout the greater length of the organ; near its tip, however, they are exarch (fig. 26). In all cases the orientation is collateral ectophloic, although at levels where branching is effected there is always an apparent concentric arrangement where the 2 xylem masses are still in contact, and the phloem masses are swung to right and left of them. Transfusion tissue accompanies the metaxylem. Mucilage ducts and tannin cells are abundant.

The root meristem is plainly visible below the hypocotyl plate in embryos of the age shown in fig. 2, but no differentiation has taken place, and of course there is no vascular tissue. The 4 poles of the root are later developed in connection with the 4 protoxylem groups of the hypocotyl vascular plate.

The plumule consists of 3 or 4 abortive scales inclosing the rudiments of the first and second true leaves, sometimes of a third leaf, and the stem tip. There is no means of distinguishing between the stem tip rudiments and those of a new leaf, because the leaf meristem grows much more rapidly, and soon overtops the stem tip (fig. 14). The vascular system supplying all these bracts and leaves is complicated by the well known habit of girdling, the details of which have fascinated and baffled many investigators. Although *D. spinulosum* differs in no way from the other cycads in this respect, its greater size makes a naked eye drawing possible and thus furnishes a means for solution and demonstration. Figs. 14 and 15 were drawn from macerated stems. Each node of the stem is, like the nodes on a first year stem of foxglove, telescoped within the older one instead of growing above it. The internodes are not elongated because the primary meristem of the stem tip is held in check by the more rapidly growing secondary meristem for each developing leaf. Since each leaf is supplied with strands from cauline bundles in different parts of the stem, those strands which come to it from the opposite side of the stem describe almost a semicircle to reach the leaf; those which arise on the same side as the leaf pass directly into it; and small arcs are described by those strands which arise in intermediate positions. In fig. 14

the traces for the older leaf (1^1) are supplied from the cauline strands numbered 2, 7, 14, 17, and 20. The strands from 2 and 20 will girdle the stem cylinder, those from 7 and 17 will make a partial girdle, and that from 14 will enter the leaf petiole directly. Fig. 15 is an attempt at demonstrating the same condition lower down in the stem. For the purpose of making the condition clearer the vertical magnification was made greater than the horizontal one.

Many angiosperms which have both "radical" and cauline leaves give illustration of this same condition. In the second year stems of such plants each node is located at some distance above the older one, and the leaf traces arising on the side of the stem opposite the leaf describe a spiral or oblique arc before entering the leaf. In the part of the stem from which the so-called "radical" leaves spring, however, the vascular strands destined for these leaves describe a horizontal arc similar to the leaf trace girdle in all parts of the cycad stem.

A careful but vain search was made for cortical cambium, vestigial traces of the primitive polystele of the Cycadofilicales.

Germination

Germination is hypogean, like that of all other members of the order thus far described. When the embryo has grown to the full length of the seed, the thin portion of the stony coat surrounding the micropyle yields to the pressure exerted upon it by the base of the axis. This base, scarcely more than a root sheath, emerges, pushing before it the brown and withered remains of the suspensor and archegonial wall. The cotyledonary base elongates and bends downward, and the root tip emerges from its sheath (fig. 3). If the embryo is a monocotyledonous one, that is, if its whole cotyledonary apparatus is a single sheath surrounding the plumule, this sheath is split by the radial growth of the axis; if there are two or more distinct cotyledons, their petioles are separated by the same cause (fig. 4). The plumule then emerges from the seed and becomes erect. Of course, when the seeds germinate while in a vertical position, the root and the plumule develop in the same axis (figs. 5, 6). In 3 of the seeds double embryos developed (fig. 29).

Seedling

The primary root persists indefinitely as a tap root. Large quantities of starch are transferred to it through the cotyledons, and it becomes large and swollen. The small lateral roots arise in whorls, usually of 4, and become a matted mass of fibers. Almost all the greenhouse grown seedlings have their roots hypertrophied, and the root tips have the characteristic tubercles described by LIFE[8] in *Cycas*.

The first leaves are yellowish scales, although thick and fleshy. They bear stipules like those of the foliage leaves, and some of them manifest the typical circinate venation (figs. 10, 11). The true leaves have been described by CHAMBERLAIN in the work previously cited. The first true leaves of several of my seedlings had as few as 6 pairs of leaflets (fig. 9), but most of them had 12 or more. There is great variation in the size of leaves of plants grown in the same conditions and from the same sized seeds. One plant bore small leaves with leaflets 2×0.3 cm., while another just beside it bore on its first leaf 12 pairs of leaflets, each one of them 6×1.1 cm. Imitation of the moist air conditions under which the first leaves of *Ceratozamia* became foliage leaves was unsuccessful with *Dioon spinulosum*.

The stele of the primary root is tetrarch, changing to diarch in its later formed portions and in the lateral branches. The relative size of the vascular cylinder to the whole root in various levels is shown by figs. 16–19. In the hypocotyl the diameter of the vascular cylinder is only about one-seventh that of the whole axis. The manner in which the radial position is achieved in the root is illustrated in figs. 18 and 19.

The leaf traces are always endarch and collateral, and their arrangement in the petiole as shown in cross-section presents the well known omega-shape. Branching and anastomosis are frequent throughout the petiole, but there is in general a diminution of traces toward the top of the leaf.

The plant is a much more rapid grower than *Dioon edule*, and is far more graceful. Under favorable conditions in the greenhouse,

[8] LIFE, A. C., The tuber-like rootlets of *Cycas revoluta*. BOT. GAZ. 31:265–271. 1901.

plants made 3 and 4 leaves, each 1 m. long and 14 cm. wide, in less than a year after emerging from seed.

Summary

1. The cotyledons of *Dioon spinulosum* vary in number from 2 to 4, and they are often lobed and divided so as to appear greater in number. In rare cases the cotyledonary sheath is undivided except near the tip.

2. They are multifascicular, like those of *Ceratozamia* and *Microcycas*, rather than like those of *Zamia* and *Cycas*, which have but few strands.

3. The arrangement and orientation of the vascular strands of cotyledons, hypocotyl, stem, leaves, and root do not differ in any marked degree from the general cycad arrangement.

4. The stem is large enough to demonstrate the cause of the girdling habit and to bring it into alignment with certain angiosperms of the same habit.

5. There is no extrafascicular cambium or any other vestige of polystyle.

Grateful acknowledgment is due to Dr. CHARLES J. CHAMBERLAIN for the generous supply of material from which this study was made.

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EXPLANATION OF PLATES X, XI

The drawings 1–13 and 29 were made with the unaided eye and are reduced to one-half the natural size; 14–19 are diagrammatic; the remainder were made with the aid of the Abbe camera lucida. The following abbreviations have been used: *c*, cotyledon; *l*, leaf; *s*, suspensor; *sc*, scale leaf; *st*, stipule; *vc*, vascular cylinder; *x*, xylem; *ph*, phloem; *px*, protoxylem; *mx*, metaxylem; *ep*, inner epidermis of the cotyledons.

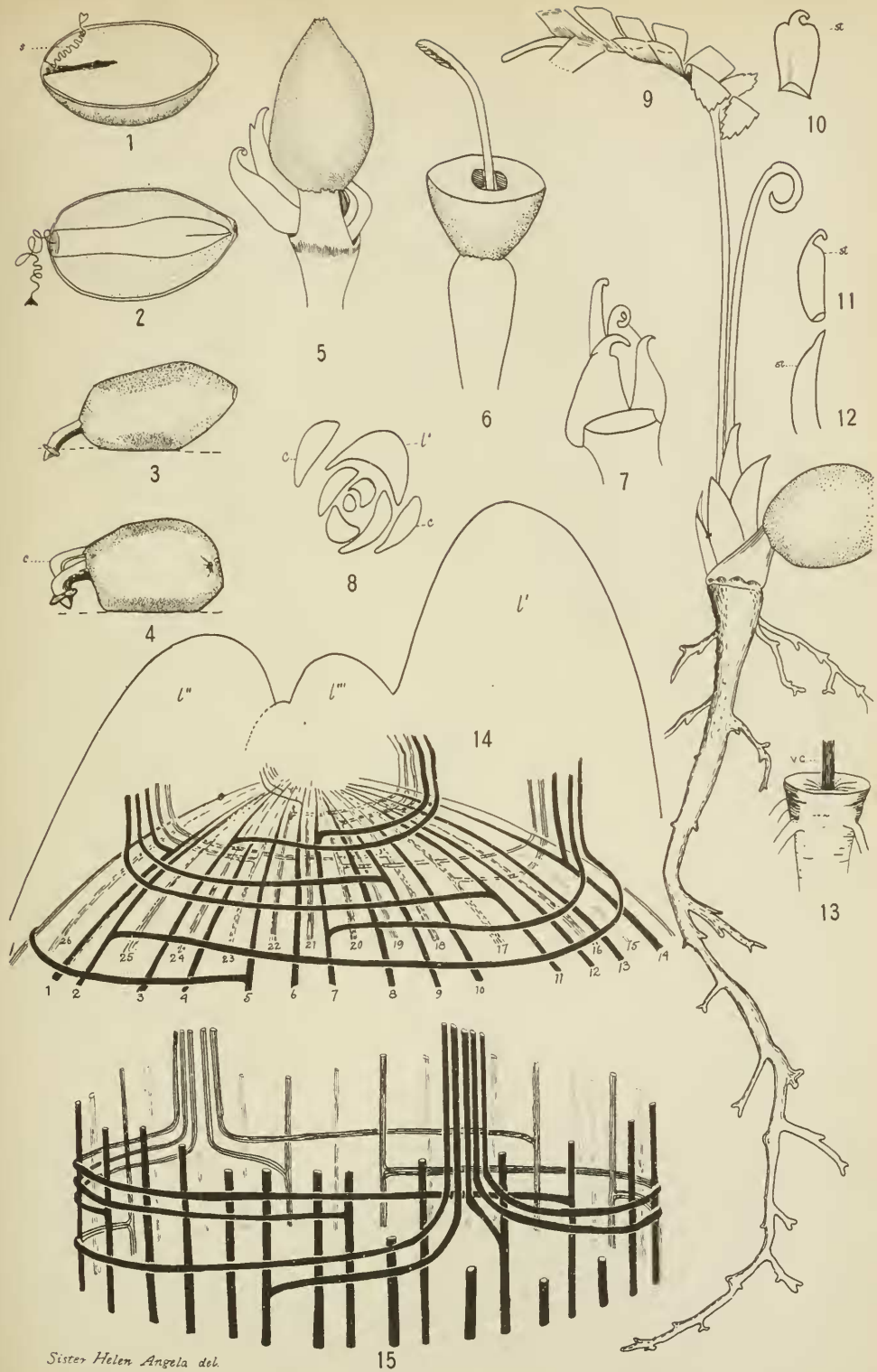
FIG. 1.—Ovule with young embryo and coiled suspensor.

FIG. 2.—Seed with mature embryo, pushing out suspensor and archegonial wall.

FIG. 3.—Beginning of germination.

FIG. 4.—Separation of cotyledonary petioles.

FIG. 5.—Seedling germinated in vertical position.



DORETY on DIOON